Article Watch: April 2018

Clive A. Slaughter

This column highlights recently published articles that are of interest to the readership of this publication. We encourage ABRF members to forward information on articles they feel are important and useful to Clive Slaughter, MCG-UGA Medical Partnership, 1425 Prince Ave., Athens, GA 30606, USA. Tel: (706) 713-2216; Fax: (706) 713-2221; E-mail: cslaught@uga.edu, or to any member of the editorial board. Article summaries reflect the reviewer's opinions and not necessarily those of the association.

NUCLEIC ACID SEQUENCING

Chen Z, Zhou W, Qiao S, Kang L, Duan H, Xie X S, Huang Y. Highly accurate fluorogenic DNA sequencing with information theory-based error correction. *Nature Biotechnology* 35;2017:1170–1178.

"Next-generation" sequencing is based on the principle of sequencing by synthesis (SBS). A DNA polymerase is used to extend a new DNA strand on a template whose sequence is deduced by detecting the nucleotides as they are incorporated. With the exception of the Pacific Biosystems single-molecule sequencing system (in which strand synthesis is monitored continuously), chain elongation is a cyclic process, and the sequence of the template stand is deduced by identifying the nucleotide incorporated into the growing chain in each synthesis cycle. SBS methodology is limited by high error rates, but Chen *et al.* here describe a new SBS implementation that greatly enhances sequencing accuracy. Their improvements are achieved by deployment of fluorescent labels with enhanced characteristics for nucleotide identification, an increase of the amount of information collected within a given sequencing reaction time to enhance base-calling confidence, and incorporation of error correction strategies derived from information communication theory. The authors have developed a new family of fluorescent sequencing substrates for fluorogenic SBS that use Tokyo Green, a dye that provides enhanced quantum yield, absorption coefficient, and on-off ratio compared with previously reported dyes. When the DNA polymerase incorporates a nucleotide, its label is released in a nonfluorescent state that is immediately switched to a highly fluorescent state by alkaline phosphatase-catalyzed dephosphorylation. The nascent strand is left with a free 3′-OH to which further nucleotides can be added, even within the same cycle. In each reaction cycle, a mixture of 2 nt is supplied, and residues are added until a mismatch with the template is encountered. Cycles alternate between two orthogonal nucleotide pairs, *e.g.*, A + C and G + T. Fluorescence intensity indicates the number of complementary nucleotides added in each cycle. This process results in a degenerate polymer sequence, which can be read accurately for 125 cycles (~250 nt). To convert the degenerate sequence into an explicit nucleotide sequence, the nascent strand is removed, the template is reannealed with primer, and the process is repeated with another set of orthogonal nucleotide pairs, *e.g.*, A + G and C + T. The explicit nucleotide sequence is then extracted by taking the intersection between the degenerate polymer sequences. For additional redundancy, 3 degenerate polymer sequences are compiled in this way (the final one with A + T and C + G in this example). Inevitably, errors arise through dephasing among the 3 degenerate polymer lengths as a result of apparent nucleotide insertions or deletions (but in this methodology, not substitutions). The errors are not just flagged but also corrected. Correction is performed by iteratively changing degenerate polymer sequences to achieve concordance within the redundant dataset. The authors have developed dynamic programming methods for sequence correction based on a model derived from the field of information communication and storage. This is the first implementation of error correction encoding in SBS. The entire methodology is named Error Correction Code (ECC) sequencing. It provides error-free read lengths of up to 200 nt and 99.96% accuracy of up to 300 nt. Further refinement is envisioned, but even at the current performance, the methodology represents a major advance in the analysis of rare genomic variations.

GLYCANS

Shajahan A, Supekar N T, Heiss C, Ishihara M, Azadi P. Tool for rapid analysis of glycopeptide by permethylation via one-pot site mapping and glycan analysis. *Analytical Chemistry* 89;2017:10734–10743.

Permethylation continues to be useful in the mass spectral analysis of the carbohydrate sidechains of glycoproteins. Glycosidic linkages can be assessed in glycosidic and

doi: 10.7171/jbt.18-2901-004

crossring fragments, as every free hydroxyl group (–OH) is replaced by a methoxy group ($–OCH_3$), whereas hydroxyl groups involved in glycosidic linkages are not methylated. Permethylation improves mass spectral sensitivity, stabilizes glycans against in-source fragmentation, and allows simultaneous analysis of basic, acidic, and neutral carbohydrates. To simplify mass spectral interpretation, the procedure is normally conducted on glycan chains from which the peptide has been completely removed by chemical or enzymatic hydrolysis. Such removal is sometimes difficult to accomplish and usually sacrifices information about the location of glycosylation sites. Although permethylation was used in the earliest days of mass spectrometric peptide sequence analysis, knowledge of peptide permethylation patterns has long been relegated to scientific archeology. The present authors, however, demonstrate that permethylation patterns of peptides are simple, limited, and predictable and show that permethylation of intact glycopeptides affords a general approach to the structural characterization and localization of glycan sidechains in glycoproteins. They provide rules for the modification of amino acids during basic permethylation and an optimized strategy for controlled peptide digestion with pronase to produce glycopeptides with manageable peptide length.

Klamer Z, Staal B, Prudden A R, Liu L, Smith D F, Boons G-J, Haab B. Mining high-complexity motifs in glycans: a new language to uncover the fine specificities of lectins and glycosidases. *Analytical Chemistry* 89;2017:12342–12350.

The structural features of glycans that primarily mediate their binding to a particular lectin or their transformation by a particular glycosyltransferase or glycosidase can be readily discerned by semiquantitative testing of the protein against a small panel of glycans. However, binding and activity may be modulated by additional, subtle features of glycan structure. For example, recognition might be governed by substituents on neighboring monosaccharides, the length of the branch containing the binding site, or the distribution of binding sites between branches. The discernment of the structural basis of such specificities typically entails quantitative measurements with extensive arrays of glycoforms. Klamer *et al.* here describe a new nomenclature syntax to describe structural motifs that putatively affect such specificity. The syntax can be read by human investigators and embraces motifs of nearly any complexity, including noncontiguous structural features of glycans. Algorithms for automated application of this syntax to the analysis of glycan array data are also provided. With the use of these methods, the authors identify previously unrecognized motifs determining binding by a lectin and discover potential fine specificity determinants for two glycosidases.

MACROMOLECULAR SYNTHESIS AND SYNTHETIC BIOLOGY

Chevalier A, Silva D-A, Rocklin G J, Hicks D R, Vergara R, Murapa P, Bernard S M, Zhang L, Lam K-H, Yao G, Bahl C D, Miyashita S-I, Goreshnik I, Fuller J T, Koday M T, Jenkins C M, Colvin T, Carter L, Bohn A, Bryan C M, Fernández-Velasco D A, Stewart L, Dong M, Huang X, Jin R, Wilson I A, Fuller D H, Baker D. Massively parallel de novo protein design for targeted therapeutics. *Nature* 550;2017:74–79.

Chevalier *et al.* exploit new capabilities for *de novo* computational design of mini-proteins and conduct experimental selection for mini-protein target binding on a massive scale to design molecules with pharmaceutical potential. For the present study, they chose influenza A H1 hemagglutinin (HA) and botulinum neurotoxin B (BoNT) as targets. The authors begin with 4000 virtual scaffolds having geometries ααα, βαββ, αββ, ββαβ, and αββα, both with and without stabilizing disulfide bonds. Residues from helices in known molecules that bind the targets are then incorporated into helical segments of the scaffolds, and surrounding residues are optimized using Rosetta combinatorial sequence optimization. They chose designs numbering 7276 against HA and 3406 against BoNT for experimental characterization, along with various control sequences. Oligonucleotide pools encoding all of the designs are synthesized, amplified, and cotransformed into yeast. The yeast libraries, which display a total of 16,968 proteins, are incubated with a fluorescently labeled target at varying concentrations. Cells displaying designs that bind the target are recovered by fluorescence-activated cell sorting and characterized by deep sequencing. At target concentrations of 10 nM, the enriched pools contain 987 BoNT binders and 41 HA binders. Designs with and without disulfides produce similar success rates, but when design libraries are incubated with trypsin before binding selection, only those stabilized with disulfides are recovered. Further biologic characterization is performed with designs chemically synthesized or expressed in *Escherichia coli*. One of the HA binders is shown to protect mice efficiently from influenza, either before or after viral exposure, at intranasal doses as low as 0.03 mg/kg. This dose is 100 times lower by mass than a dose of the neutralizing antibody, FI6v3, required for equivalent protection. Importantly, the mini-proteins are found to be minimally immunogenic, presumably because their molecular size is small and their structure rigid. It is hoped that designed mini-proteins will contribute to both diagnostic and therapeutic procedures in the future.

METABOLOMICS

Schuhmann K, Srzentić K, Nagornov K O, Thomas H, Gutmann T, Coskun Ü, Tsybin Y O, Shevchenko A. Monitoring membrane lipidome turnover by metabolic ^{15}N labeling and shotgun ultra-high-resolution Orbitrap Fourier transform mass spectrometry. *Analytical Chemistry* 89;2017:12857–12865.

Although high-throughput methods for identifying and quantifying lipids are now available, the task of measuring their turnover remains technically challenging. Metabolic incorporation of ^{13}C from glucose or acetate is seldom complete because of the large numbers of carbon atoms per molecule, and the method excludes lipids containing essential fatty acids. Targeted deuterium labeling of class-specific head groups is possible but is not an approach conducive to broad lipidome coverage, and incorporation of multiple deuterium atoms can affect the way lipids interact with one another or with proteins. Schuhmann *et al.* here describe a methodology for measurement of lipid turnover that is compatible with broad-based shotgun lipid profiling and quantification. They use ^{15}N labeling. Many major classes of the membrane lipid have head groups containing 1 nitrogen atom (*e.g.*, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and sphingolipids) or 2 nitrogens (*e.g.*, sphingomyelin and some glycosphingolipids). The nominal masses of lipids labeled with one ^{15}N are, of course, indistinguishable from the first isotopic peak of the unlabeled species, but at ultrahigh resolution, ^{15}N-labeled species are distinguishable by their mass defect. Therefore, the authors conduct their analyses on an Orbitrap Elite mass spectrometer which, operating at a resolution of 1.35×10^6 (at m/z 200), achieves baseline separation of the ^{15}N-labeled molecules within the isotopic cluster (Δm = 0.0063 Da). They document lipidome composition and fluxes of 62 nitrogen-containing lipids in human HepG2 cells.

Warth B, Spangler S, Fang M, Johnson C H, Forsberg E M, Granados A, Martin R L, Domingo-Almenara X, Huan T, Rinehart D, Montenegro-Burke J R, Hilmers B, Aisporna A, Hoang L T, Uritboonthai W, Benton H P, Richardson S D, Williams A J, Siuzdak G. Exposome-scale investigations guided by global metabolomics, pathway analysis, and cognitive computing. *Analytical Chemistry* 89;2017:11505–11513.

The task of measuring human exposure to the panoply of chemicals and toxins encountered in the environment is fundamentally important for epidemiology, healthcare, and preventative medicine. Comprehensive measurement of the levels of environmental chemicals in physiologic fluids is nevertheless extremely challenging. The levels of these chemicals and their metabolites are often very low, yet range over orders of magnitude for different classes of compounds. Comprehensive analytical strategies have not been established, and relevant mass spectral databases are incomplete. The authors here present an untargeted metabolomics workflow for identification of xenobiotics based on liquid chromatography–high-resolution mass spectrometry. They expand the METLIN database to 950,000 chemicals, which include drugs, toxicants, contaminants, lipids, sterols, small peptides, carbohydrates, and central carbon metabolites. The database is interrogated with the cloud-based metabolomics analysis platform XCMS Online, which supports pathway analysis. The authors believe that their methodology will also find application in related fields of food, feed, water, air, and soil analysis.

PROTEOMICS

Frost D C, Buchberger A R, Li L. Mass defect-based dimethyl pyrimidinyl ornithine (DiPyrO) tags for multiplex quantitative proteomics. *Analytical Chemistry* 89;2017:10798–10805.

Frost *et al.* take a new look at mass defect methodology for multiplex peptide quantification, first introduced by J. Coon's group in 2013–14 (Herbert A S, *et al. Nature Methods* 10;2013:332–334). Samples are labeled with tags differing in mass in the mDa range. Such small mass differences can be distinguished at high resolution without contributing to spectral complexity at low resolution. The methodology permits quantification based on precursor ion (MS1) scans, thereby avoiding distortion of abundance ratios as a result of precursor coisolation that is encountered in quantification based on product ion (MS2) scans. The amine-reactive tags originally described by Coon's group are large (431 Da), they tend to inhibit sequence-informative fragmentation, and they contribute to sequence-uninformative fragmentation. Frost *et al.* here describe the design, synthesis, and application of a set of mass defect-based tags that are smaller (contributing only 254 Da to the labeled peptide) and support enhanced ionization and informative fragmentation. These characteristics lead to more confident sequence identification. The mass defect signatures incorporated into the tags support 2-plex analysis at 100,000 resolution and 3- or 6-plex analysis at 240,000 and 480,000 resolution, respectively. The authors hope that the new tags will encourage wider application of mass defect quantification in proteomics.

FUNCTIONAL GENOMICS AND PROTEOMICS

Abudayyeh O O, Gootenberg J S, Essletzbichler P, Han S, Joung J, Belanto J J, Verdine V, Cox D B, Kellner M J, Regev A, Lander E S, Voytas D F, Ting A Y, Zhang F. RNA targeting with CRISPR-Cas13. *Nature* 550;2017:280–284.

Cox D B, Gootenberg J S, Abudayyeh O O, Franklin B, Kellner M J, Joung J, Zhang F. RNA editing with CRISPR-Cas13. *Science* 358;2017:1019–1027.

Methods for genomic DNA base editing that use the clustered regularly interspaced short palindromic repeats-caspase 9 (CRISPR-Cas9) system have found widespread application, but comparably convenient methods for targeting single-stranded mRNA have not been described previously. Although most CRISPR-associated Cas nucleases target DNA substrates, there exist CRISPR systems that target RNA. Two papers now exploit RNA-cleaving Cas enzymes for sequence-specific RNA engineering. Abudayyeh *et al.* investigate one such enzyme, Cas13, an RNA-programmable RNAse that can cleave single-stranded RNA with a protospacer complementary to a small guide RNA. Unlike Cas9, however, it does not require a protospacer-adjacent motif to recognize its target. The authors screen orthologs of Cas13 for RNA knockdown activity and identify Cas13a from the bacterium *Leptotrichia wadei* (LwaCas13a) as the ortholog most effective in *Escherichia coli*. They show that this enzyme can be expressed in mammalian and plant cells and can knock down either reporter or endogenous transcripts as efficiently as the knockdown achieved by RNA interference by short hairpin RNAs, and with greater specificity. They also show that a catalytically inactive LwaCas13a, fused with green fluorescent protein and a nuclear localization sequence, can be used to track specific transcripts in live cells. Cox *et al.* further develop this methodology by screening additional codon-optimized Cas13 orthologs for better RNA interference activity in mammalian cells and identify one from the bacterium *Prevotella* (PspCas13b). They fuse this protein with the catalytic domain of adenosine-to-inosine deaminase 2 to perform programmable A-to-I replacement in mRNA. The inosine residues are equivalent to guanosine in translation and splicing. The authors further engineered the system to increase its specificity, and they minimized it to facilitate viral delivery. The result is a methodology for correcting full-length transcripts containing pathogenic mutations without sequence constraints, although with modest efficiency. Such RNA targeting reduces anxiety engendered by methods that permanently alter heritable genomic sequences. The circumscribed duration of activity in cells well suits the methodology for remediating transient conditions, such as localized inflammation.

GTEx Consortium. Genetic effects on gene expression across human tissues. *Nature* 550;2017:204–213.

Li X, Kim Y, Tsang E K, Davis J R, Damani F N, Chiang C, Hess G T, Zappala Z, Strober B J, Scott A J, Li A, Ganna A, Bassik M C, Merker J D, Consortium G T, Hall I M, Battle A, Montgomery S B. The impact of rare variation on gene expression across tissues. *Nature* 550;2017:239–243.

In a series of papers, the Genotype-Tissue Expression Consortium (GTEx) outlines progress in an ongoing project to identify human expression quantitative trait loci (eQTL) that are correlated with variation in the expression of genes in different tissues. In the latest phase of the project, the consortium combines gene-expression data from 44 tissues derived from a total of 449 recently deceased individuals. In the first paper considered here, the consortium shows that genetic variation affects the expression of most genes in the genome. Variants situated close to the affected gene typically exert their effects in *cis* and influence expression of their target gene in the majority of tissues. Variants situated farther away exert their effects in *trans* and influence expression in 1 or a few tissues. In the second paper, Li *et al.* present statistical methodology for associating rare variants with gene-expression changes of large magnitude and conclude that rare variants contribute importantly to gene-expression differences among individuals in the population. Participants in the project hope to extend the study to identify eQTL affecting gene expression in single cells of a defined type and, with the use of experimental DNA editing approaches, to establish causal relationships between identified genes and expression differences.

Elling U, Wimmer R A, Leibbrandt A, Burkard T, Michlits G, Leopoldi A, Micheler T, Abdeen D, Zhuk S, Aspalter I M, Handl C, Liebergesell J, Hubmann M, Husa A-M, Kinzer M, Schuller N, Wetzel E, van de Loo N, Martinez J A Z, Estoppey D, Riedl R, Yang F, Fu B, Dechat T, Ivics Z, Agu C A, Bell O, Blaas D, Gerhardt H, Hoepfner D, Stark A, Penninger J M. A reversible haploid mouse embryonic stem cell biobank resource for functional genomics. *Nature* 550;2017:114–118.

Elling *et al.* announce a resource of over 100,000 independent, haploid, mouse embryonic stem cell lines containing mutations in a total of 16,970 genes, which represent over 70% of the protein-coding genes in the mouse genome. This library is derived from AN3-12 cells, which do not require feeder cells and maintain a stable haploid genome

that enables recessive mutations to be investigated. These cells remain undifferentiated in culture but express pluripotency markers and differentiate into all germ layers *in vivo*. The mutations have been generated by insertional mutagenesis using barcoded vectors. Invertable splice acceptor sites allow repair of the targeted genes so that gene function can be assessed in sister cells despite clonal variation. The high proportion of genes covered by the library enables both forward genetic screening to discover novel genes mediating functions of interest and reverse genetic screening to investigate the function of particular genes.

IMAGING

Zheng W, Wu Y, Winter P, Fischer R, Nogare D D, Hong A, McCormick C, Christensen R, Dempsey W P, Arnold D B, Zimmerberg J, Chitnis A, Sellers J, Waterman C, Shroff H. Adaptive optics improves multiphoton superresolution imaging. ***Nature Methods*** **14;2017:869–872.**

Gregor I, Spiecker M, Petrovsky R, Grosshans J, Ros R, Enderlein J. Rapid nonlinear image scanning microscopy. ***Nature Methods*** **14;2017:1087–1089.**

In these two papers, improvements are made to image scanning microscopy (ISM), a super-resolution implementation of fluorescence microscopy capable of doubling the spatial resolution relative to Abbé's limit. Both groups combine the technique with 2-photon excitation microscopy, a method that enables scanning to depths of hundreds of micrometers in thick tissue samples with excellent background rejection. Zheng *et al.* correct for aberration by scanning over the sample using a wavefront sensor to characterize distortions and then applying a compensatory wavefront to a deformable mirror, thereby correcting aberrations in both the excitation and emission arms of the system. In this way, they achieve images with lateral resolution of 176 nm and axial resolution (sectioning capability) of 729 nm at tissue depths of ~250 μm. Gregor *et al.* describe a way to simplify the instrumentation needed for 2-photon ISM. Instead of the two synchronized scanners normally used for rescanning to achieve super-resolution in "all-optical" ISM (one scanner to scan the excitatory beam across the sample and the other to increase the sweep area of the camera), Gregor *et al.* achieve similar results with just one scanner. A scan- and tube-lens pair relays the excitation beam from the pivot point of the scanner to the back focal plane of the microscope objective. Emitted light from the sample is relayed in the reverse direction: it is deflected by a dichroic filter and then projected by a second scan- and tube-lens pair back to the entrance port of the same scanner. The rescanned, emitted light is coupled out by a dichroic mirror, passes through a band-pass filter, and is focused by an imaging lens into a camera. The authors also demonstrate the system in second harmonic-generation mode. Their system provides better sensitivity and higher frame rates than existing systems and can image to a depth of at least 100 μm. It can be implemented on existing microscopes.

Grimm J B, Muthusamy A K, Liang Y, Brown T A, Lemon W C, Patel R, Lu R, Macklin J J, Keller P J, Ji N, Lavis L D. A general method to fine-tune fluorophores for live-cell and ***in vivo*** **imaging.** ***Nature Methods*** **14;2017:987–994.**

The authors' laboratory has previously described a method for synthesizing rhodamine that can support the production of derivatives of the rhodamine skeleton with diverse substituents useful for varying purposes. With the use of this method, they produced a new dye, Janelia Fluor 549 (JF_{549}), which contains an azetidine ring (a 4-member ring containing 3 carbon atoms and 1 nitrogen atom) and shows improved brightness and photostability. In the present paper, they design and test substituents at the 3 position of the azetidine ring in JF_{549} to modulate λ_{abs}, λ_{em}, and the equilibrium between the colorless, nonfluorescent "closed" lactone form of the molecule and the colored, fluorescent, "open" zwitterionic form, without affecting fluorescence quantum yield. The work has resulted in 4 new fluorophores, fine-tuned for use with standard laser excitation sources across the visible spectrum. The tuning rules discovered in the course of the experiments are generalizable to other classes of fluorophores. The new compounds are immediately applicable for structured illumination and stimulated emission depletion applications. The methods that produced the new fluorophores are anticipated to be useful for the synthesis of further derivatives that share high quantum yield yet display photophysical and chemical properties fine-tuned for a broad range of specific biologic imaging experiments.

BACTERIA

Costea P I, Zeller G, Sunagawa S, Pelletier E, Alberti A, Levenez F, Tramontano M, Driessen M, Hercog R, Jung F-E, Kultima J R, Hayward M R, Coelho L P, Allen-Vercoe E, Bertrand L, Blaut M, Brown J R, Carton T, Cools-Portier S, Daigneault M, Derrien M, Druesne A, de Vos W M, Finlay B B, Flint H J, Guarner F, Hattori M, Heilig H, Luna R A, van Hylckama Vlieg J, Junick J, Klymiuk I, Langella P, Le Chatelier E, Mai V, Manichanh C, Martin J C, Mery C, Morita H, O'Toole P W, Orvain C, Patil K R, Penders J, Persson S, Pons N, Popova M, Salonen

A, Saulnier D, Scott K P, Singh B, Slezak K, Veiga P, Versalovic J, Zhao L, Zoetendal E G, Ehrlich S D, Dore J, Bork P. Toward standards for human fecal sample processing in metagenomic studies. *Nature Biotechnology* 35;2017:1069–1076.

Sinha R, Abu-Ali G, Vogtmann E, Fodor A A, Ren B, Amir A, Schwager E, Crabtree J, Ma S, The Microbiome Quality Control Project C, Abnet C C, Knight R, White O, Huttenhower C. Assessment of variation in microbial community amplicon sequencing by the Microbiome Quality Control (MBQC) Project Consortium. *Nature Biotechnology* 35;2017:1077–1086.

Variability in measurements of the taxonomic composition of commensal microbiota that results from methodological and interlaboratory differences may exceed genuine biologic variation and, therefore, affects data interpretation. Two papers consider the sources of systematic variation in microbiome studies. Costea *et al.* conclude that DNA extraction has the largest impact on results. They compare DNA extraction methods in common use for shotgun metagenomic analysis of human fecal samples, assess reproducibility within and between laboratories, and recommend a standard methodology involving DNA extraction protocol Q, which is used in the Qiagen QIAamp DNA Stool Kit. Sinha *et al.* report the results of a microbiome quality-control project involving 15 laboratories that perform 16S rRNA sequencing of human fecal samples. They concluded that variability depends most on specimen type and origin, followed by the DNA extraction method, sample handling (including choice of 16S rRNA gene variable primers), and bioinformatic methodology. Both groups use reference standards representing mock microbial communities of known composition and concur that adoption of such standards and use of them to normalize results and control for contamination among samples (another important source of variability) will be helpful in the field.

CELL BIOLOGY AND TISSUE ENGINEERING

Jun J J, Steinmetz N A, Siegle J H, Denman D J, Bauza M, Barbarits B, Lee A K, Anastassiou C A, Andrei A, Aydın Ç, Barbic M, Blanche T J, Bonin V, Couto J, Dutta B, Gratiy S L, Gutnisky D A, Häusser M, Karsh B, Ledochowitsch P, Lopez C M, Mitelut C, Musa S, Okun M, Pachitariu M, Putzeys J, Rich P D, Rossant C, Sun W-l, Svoboda K, Carandini M, Harris K D, Koch C, O'Keefe J, Harris T D. Fully integrated silicon probes for high-density recording of neural activity. *Nature* 551;2017:232–236.

The recording of activity of individual neurons is most commonly performed using extracellular microelectrodes. The coordinated activity of neuronal populations in the brain is amenable to investigation with electrodes that incorporate probes with an array of recording sites. Each site may detect spikes of activity from several neurons, but the spacing between sites is close enough to allow the spikes from individual neurons to be distinguished. Jun *et al.* have fabricated probes that increase, by an order of magnitude, the number of neurons that can be simultaneously recorded in this way, while maintaining low noise levels. Their new design of probe, called *Neuropixels*, contains 960 recording sites on a single shank, spaced 20 μm apart. Simultaneous recording from 384 of these sites can be selected at any one time, and the channels being monitored can be switched to allow recording at different sites after implantation. The shank is 1 cm long—long enough to penetrate to the deepest levels of a rodent brain. Its cross-section is 70 × 20 μm—thin enough to limit cellular damage during insertion. Electronics for amplification, multiplexing, and digitizing of neural signals are incorporated into the probe base to reduce interference that results from motion artifacts. This allows recording from freely moving animals. The implants are monitored chronically for a period of at least 8 wk. With the use of 2 probes, the authors monitor >700 well-isolated single neurons. As temporal resolution is excellent (submillisecond), neurons that are functionally connected can be identified: if 1 neuron consistently fires immediately after another, then a direct connection between them may be inferred. In this way, the coordinated activity of neurons in multiple brain regions may be monitored. This technology represents a major advance in capability for observation of neural circuitry and opens opportunities to correlate behavioral changes with neural activity. With the incorporation of optogenetic techniques, the effects of specific cell types on larger circuits may also be ascertained.

DRUG DISCOVERY AND SYNTHESIS

Klaeger S, Heinzlmeir S, Wilhelm M, Polzer H, Vick B, Koenig P-A, Reinecke M, Ruprecht B, Petzoldt S, Meng C, Zecha J, Reiter K, Qiao H, Helm D, Koch H, Schoof M, Canevari G, Casale E, Depaolini S R, Feuchtinger A, Wu Z, Schmidt T, Rueckert L, Becker W, Huenges J, Garz A-K, Gohlke B-O, Zolg D P, Kayser G, Vooder T, Preissner R, Hahne H, Tõnisson N, Kramer K, Götze K, Bassermann F, Schlegl J, Ehrlich H-C, Aiche S, Walch A, Greif P A, Schneider S, Felder E R, Ruland J, Médard G, Jeremias I, Spiekermann K, Kuster B. The target landscape of clinical kinase drugs. *Science* 358;2017:eaan4368.

There are now 37 approved small-molecule protein kinase inhibitors, of which imatinib is the prototype. At least

250 more are presently undergoing clinical evaluation. Most of these compounds target more than one protein. Their off-target interactions not only determine undesired side-effects but also define scope for repurposing. The present paper characterizes the target space for 234 kinase inhibitors that are either approved or under test in humans for use in drug discovery and clinical decisionmaking. The authors use kinobeads to recover interacting proteins from tumor cells, elute the proteins from the beads, digest with trypsin, and identify them by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The target space, selectivity, and full dose-response characteristics are documented for each inhibitor. The 220 kinases identified to bind with submicromolar affinity constitute the presently druggable kinome. The authors illustrate the application of their dataset by the new identification of inhibitors capable of modulating specific processes, by identifying a drug with putative activity against a specific lung cancer biomarker, and by the discovery of a candidate for repurposing to treat acute myeloid leukemia.

POLICY

Wolpe P R, Rommelfanger K S, the Drafting and Reviewing Delegates of the BEINGS Working Groups. Ethical principles for the use of human cellular biotechnologies. *Nature Biotechnology* 35; 2017:1050–1058.

As stakeholders in the practice of biotechnology, readers of this journal may wish to become familiar with discussions surrounding the principles upon which decisions about development and deployment of specific technologies are based. Unanimity does not presently exist. For example, 40 countries have enacted laws against human gene editing for reproductive purposes (although the United States has not), yet China, Sweden, the United Kingdom, and Japan have approved gene editing of human embryos for basic scientific research (although the U.S. NIH has issued a moratorium on the funding of such research). How should support for the pursuit of knowledge be balanced with the practical application of the findings while upholding human health, social justice, human rights, protection of the natural world, and cultural diversity? Wolpe *et al.* propose 12 ethical principles for consideration, giving special consideration to gene editing and synthetic biology. Their intention is to encourage and support progress in biotechnology while protecting individuals, communities, and the environment and reflecting shared values. The authors also point out that with the increasing tendency to commercialize and privatize the products of technology, biotechnology researchers should view efforts to improve scientific literacy of potential consumers of technology as an obligation. Literacy enables citizens to contribute helpfully to discussions regarding policies and regulations.